

# SEQUENCE LISTINGS

<110> Hanmi Pharm. Co., Ltd.

<120> EXPRESSION VECTOR FOR SECRETING ANTIBODY FRAGMENT USING E. COLI SIGNAL SEQUENCE AND METHOD FOR MASS-PRODUCING ANTIBODY FRAGMENT

<130> Q94300

<140> 10/576,068

<141> 2006-04-14

<150> KR1020030072216

<151> 2003-10-16

<150> PCT/KR04/02625

<151> 2004-10-14

<160> 36

<170> KopatentIn 1.71

<210> 1

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 1

gggaagcttc gatcggacat ccagatgacc cagtctccat cctccctgtc tgcattctgta 60

ggggacagag tcacc 75

<210> 2

<211> 80

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 2

tggtttttgc tgataccagg ctaagtaatt tctgatgccc tgacttgccc gacaagtgat 60

ggtgactctg tcccctacag 80

<210> 3

<211> 80

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 3  
cctggtatca gcaaaaacca gggaaagccc ctaagctcct gatctatgct gcatccactt 60  
tgcaatcagg ggtcccatct 80

<210> 4  
<211> 80  
<212> DNA  
<213> Artificial Sequence  
<220>  
<223> gene fragment of light chain variable region

<400> 4  
aggctgtagg ctgctgatgg tgagagtga atctgtccca gatccactgc cactgaaccg 60  
agatgggacc cctgattgca 80

<210> 5  
<211> 80  
<212> DNA  
<213> Artificial Sequence  
<220>  
<223> gene fragment of light chain variable region

<400> 5  
ccatcagcag cctacagcct gaagatggtg caacttatta ctgtcaaagg tataaccgtg 60  
caccgataac ttttggccag 80

<210> 6  
<211> 41  
<212> DNA  
<213> Artificial Sequence  
<220>  
<223> gene fragment of light chain variable region

<400> 6  
tttgatttcc accttggtcc cctggccaaa agtatacggt g 41

<210> 7  
<211> 75  
<212> DNA  
<213> Artificial Sequence  
<220>  
<223> gene fragment of heavy chain variable region

<400> 7  
gggaagcttc gatcggaggt gcagctgggtg gagtctgggg gaggcttggt acagcccggc 60  
aggtccctga gactc 75

<210> 8  
<211> 79  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> gene fragment of heavy chain variable region

<400> 8  
agcttgccgg acccagtgc tggcataatc atcaaagggtg aatccagagg ccgcacagga 60  
gagtctcagg gacctgccg 79

<210> 9  
<211> 80  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> gene fragment of heavy chain variable region

<400> 9  
tgcactgggt cgggcaagct ccagggaagg gcctggaatg ggtctcagct atcacttgga 60  
atagtgggtca catagactat 80

<210> 10  
<211> 80  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> gene fragment of heavy chain variable region

<400> 10  
atacagggag ttcttggcgt tgtctctgga gatgggtgaat cggccctcca cagagtccgc 60  
atagtctatg tgaccactat 80

<210> 11  
<211> 80  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> gene fragment of heavy chain variable region

<400> 11  
 acgccaaagaa ctccctgtat ctgcaaatga acagtctgag agctgaggat acggccgtat 60  
 attactgtgc gaaagtctcg 80

<210> 12  
 <211> 84  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> gene fragment of heavy chain variable region

<400> 12  
 cactcgagac ggtgaccagg gtaccttggc cccaatagtc aagggaggac gcggtgctaa 60  
 ggtacgagac tttcgcacag taat 84

<210> 13  
 <211> 39  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> RT-PCR forward primer specific for heavy chain

<400> 13  
 cccaagctta ggcctccacc aagggcccat cggctcttc 39

<210> 14  
 <211> 48  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> RT-PCR reverse primer specific for heavy chain

<400> 14  
 gggggatcct tatgggcacg gtgggcatgt gtgagttttg tcacaaga 48

<210> 15  
 <211> 42  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> RT-PCR forward primer specific for light chain

<400> 15

cccaagcttt cgcgaactgt ggctgcacca tctgtcttca tc 42

<210> 16  
<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> RT-PCR reverse primer specific for light chain

<400> 16  
cccggatccc taacactctc ccctgttgaa gctctttgtg ac 42

<210> 17  
<211> 69  
<212> DNA  
<213> modified E. coli thermostable enterotoxin II signal sequence

<400> 17  
atgaaaaaga caatcgcatt tcttcttgca tctatgttcg ttttttctat tgctacaaat 60  
gcccaggcg 69

<210> 18  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer containing StuI restriction enzyme site

<400> 18  
tctattgcta caaatgccca ggccttccca accattccct tatcc 45

<210> 19  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> reverse primer containing StuI restriction enzyme site

<400> 19  
agataacgat gtttacgggt ccggaagggt tggttaaggga atagg 45

<210> 20  
<211> 51  
<212> DNA  
<213> Artificial Sequence

<220>

<223> reverse primer specific for light chain

<400> 20  
gggggatcct caccgggcgc atgtgtgagt tttgtcacia gatttaggct c 51

<210> 21  
<211> 43  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer containing SD sequence and BamHI restriction enzyme site

<400> 21  
gggggatcca ggaggtgatt tatgaaaaag acaatcgcat ttc 43

<210> 22  
<211> 44  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer containing BpuI restriction enzyme site

<400> 22  
ggggctgagc aggaggtgat ttatgaaaaa gacaatcgca tttc 44

<210> 23  
<211> 52  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> reverse primer containing BpuI restriction enzyme site

<400> 23  
ggggctcagc tcacggggcg catgtgtgag ttttgtcaca agatttaggc tc 52

<210> 24  
<211> 63  
<212> DNA  
<213> E. coli OmpA signal sequence

<400> 24  
atgaaaaaga cagctatcgc gattgcagtg gcactggctg gtttcgctac cgttgcgcaa 60

gct 63

<210> 25  
<211> 30

<212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> forward primer specific for heavy chain  
  
 <400> 25  
 gaggttcagc tagtcgagtc aggaggcggc 30  
  
 <210> 26  
 <211> 51  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> forward primer containing HindIII and StuI restriction enzyme sites  
  
 <400> 26  
 gggagatctt cacgcggcgc atgtgtgagt tttgtcaca gatttaggct c 51  
  
 <210> 27  
 <211> 30  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> reverse primer containing stop codon and BamHI restriction enzyme site  
  
 <400> 27  
 gacattcaaa tgaccagag cccatccagc 30  
  
 <210> 28  
 <211> 42  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> forward primer containing HindIII and NruI restriction enzyme sites  
  
 <400> 28  
 cccagatctc taacactctc ccctgttgaa gctctttgtg ac 42  
  
 <210> 29  
 <211> 41  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 29  
ggggtcgaca ggaggtgatt tatgaaaaag acagctatcg c 41

<210> 30  
<211> 51  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> reverse primer containing SalI restriction enzyme site

<400> 30  
ggggtcgact cacgcggcgc atgtgtgagt tttgtcacia gatttaggct c 51

<210> 31  
<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer specific for modified E. coli enterotoxin II signal peptide and containing NdeI restriction enzyme site

<400> 31  
gggcatatga aaaagacaat cgcatttctt cttgcatcta tg 42

<210> 32  
<211> 705  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> TNF-alpha heavy chain

<400> 32  
gaggttcagc tagtcgagtc aggaggcggg ttggtacagc ccggcaggtc cctgagactc 60  
tcctgtgcgg cctctggatt cacctttgat gattatgcca tgcactgggt ccggcaagct 120  
ccagggaagg gcctggaatg ggtctcagct atcacttgga atagtgggtca catagactat 180  
gcggactctg tggagggccg attcaccatc tccagagaca acgccaagaa ctccctgtat 240  
ctgcaaatga acagtctgag agctgaggat acggccgtat attactgtgc gaaagtctcg 300  
taccttagca ccgcgtctc ccttgactat tggggccaag gtaccctggg caccgtctcg 360  
agtgcctcca ccaagggccc atcgggtctc ccctggcac ctcctccaa gagcacctct 420  
gggggacag cgccctggg ctgcctgggc aaggactact tccccgaacc ggtgacggtg 480  
tcgtggaact caggcgccct gaccagcggc gtgcacacct tcccggctgt cctacagtcc 540



tcaggactct actccctcag cagcgtggtg accgtgccct ccagcagctt gggcaccag	600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag	660
cccaaattctt gtgacaaaac tcacacatgc ccaccgtgcc catag	705

<210> 33  
 <211> 645  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> TNF-alpha light chain

<400> 33	
gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtagggga cagagtcacc	60
atcacttgtc gggcaagtca gggcatcaga aattacttag cctgggatca gcaaaaacca	120
gggaaagccc ctaagctcct gatctatgct gcatccactt tgcaatcagg ggtcccatct	180
cggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag cctacagcct	240
gaagatggtg caacttatta ctgtcaaagg tataaccgtg caccgtatac ttttggccag	300
gggaccaagg tggaatcaa acgaactgtg gctgcacat ctgtcttcat cttcccgcga	360
tctgatgagc agttgaaatc tggaactgcc tctgttggtg gcctgctgaa taacttctat	420
cccagagagg ccaaagtaca gtggaagggtg gataacgccc tccaatcggg taactcccag	480
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg	540
ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc	600
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag	645

<210> 34  
 <211> 7  
 <212> PRT  
 <213> TNF-alpha light chain

<400> 34  
 Asp Ile Gln Met Thr Gln Ser  
 1 5

<210> 35  
 <211> 8  
 <212> PRT  
 <213> TNF-alpha heavy chain

<400> 35  
 Glu Val Gln Leu Glu Val Asp Ser  
 1 5

```

<210>      36
<211>      12
<212>      PRT
<213>      N-terminal sequence of recombinant TNF-alpha

<400>      36
Asp Glu Ile Val Gln Met Leu Thr Val Gln Asp Ser
 1              5              10

```